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Characteristics of Klebsiella from Textile Finishing Plant Effluents

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16. ABSTRACT

A survey of bacteria in a stream receiving wastes from a textile finishing plant revealed an abnormally high number of Klebsiella in the absence of any source of fecal pollution. The density at times reached as high as 1 x 10^7 cells per milliliter. Since this genus constitutes a part of the coliform group that is used as an index of fecal pollution and since some of its members are recognized as opportunistic pathogens, a more extensive study of randomly selected strains was carried out. Forty-nine isolates were characterized relative to their biochemical reactions, serotypes and pathogenicity for mice. All isolates conformed to the commonly accepted biochemical reaction scheme for Klebsiella. Of the strains serotyped, about one-fourth were found to be types commonly associated with human infections. Similarly, about one-fifth of the isolates examined were pathogenic for mice. These findings suggest first, that waters receiving wastes from textile finishing plants may present a false indication of human fecal pollution and second, these waters may be a reservoir of potential pathogens for those individuals predisposed to infection by Klebsiella species.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
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Characteristics of Klebsiella from textile finishing plant effluents

A. P. DUFOUR AND VICTOR J. CABELLI

COLIFORM BACTERIA traditionally have been used to indicate fecal contamination of water and, thus, the probable presence of enteric pathogens therein. The assumption that the density of coliforms is directly and quantitatively related to the degree of fecal pollution has led to the use of coliform criteria and standards for the sanitary quality of water. The coliform group is very heterogeneous and contains biotypes not related to fecal sources, however. These nonfecal coliform biotypes, frequently associated with stormwater runoff,[1] have a tendency to inflate the coliform count and lead to overestimation of the potential health hazard. Conversely, the inclusion in the coliform group of organisms not associated with feces probably has led to inflated numerical criteria and standards. Thus, when all of the coliforms are derived from fecal sources, an underestimate of a potential health hazard may occur.

One solution to the above problem was the use of a more restricted portion of the coliform population, specifically that portion associated more exclusively with the fecal wastes of warm-blooded animals. Use of this group led to the development and use of a test for those coliforms that produce gas from lactose in a specified medium incubated at 44.5°C, that is, "fecal coliforms."[2,3] There can be no doubt of the superiority of fecal coliforms over total coliforms as indicators of fecal pollution. A number of investigators have presented evidence demonstrating that high densities of fecal coliforms are present in waters that have not received human or animal wastes, however.[4] The coliforms most responsible for this pseudo indication of fecal pollution are members of the genus *Klebsiella*. Bauer[5] found coliform densities as high as 16×10^7/100 ml and fecal coliform densities up to 4.2×10^6 in the pulp and paper mill effluents that he examined. Of the 300 fecal coliforms that he isolated, 68 percent had a −−++ IMViC pattern; of these, 93 percent were *K. pneumoniae*. Knittel,[6] who found similar densities of coliforms in pulp and paper mill effluents, reported that 90 to 100 percent of his isolates were fecal coliforms. Dutka[7] showed that *Klebsiella* was the dominant genus in the slime from pulp and paper processing wastes. Nunez and Colmer[8] found that 86 percent of the lactose fermenting isolates from sugar cane wastes were *Klebsiella*.

Some reports that indicate that the members of the genus *Klebsiella* may be ubiquitous in nature. Duncan and Razzell[9] reported finding these organisms in the virgin forests of British Columbia, Can., and in produce from the counters of Vancouver supermarkets. Seventy-five percent of the former isolates and thirty-five percent of the latter gave positive fecal coliform tests. Brown and Seidler[10] isolated *Klebsiella* from the surfaces of vegetables and seeds, as well as from living fir trees; however, they did not subject their isolates to the fecal coliform test.

The above findings would suggest that *Klebsiella* has the ability to survive and even multiply in environments rich in polysaccharides, such as pulp mill and sugar cane wastes. Textile finishing plant wastes also fit in this category. Before the weaving process, hardeners (such as starch, carboxymethyl cellulose, and polyvinyl alcohols) are added to the thread to strengthen it. The hardeners are removed enzymatically before the dyeing process begins, and these degradation products make

up a major component of the plant waste. These wastes typically are lagooned before reaching a river or some other body of water, or they may be treated in biological degradation tanks seeded with wastewater and then passed through aeration and activated charcoal towers. The similarity of textile finishing plant effluents to pulp mill or sugar cane mill wastes suggests that some of the difficulties encountered in correctly identifying the origin and significance of coliforms from the latter wastes would also be experienced with those coming from the former. If such is the case, it has not been reported in the literature.

Aside from its possible significance as a coliform or even a fecal coliform indicator of potential health effects from fecal contamination, *Klebsiella* also is a pathogen in its own right.[11-14] This consequence of the presence of large numbers of *Klebsiella* from industrial effluents has not been clearly established. Brown and Seidler [10] did not find environmental and clinical isolates dissimilar with regard to their biochemical and serological characteristics, nor was there an appreciable difference in mouse pathogenicity among environmental isolates from sugar cane wastes when compared with those from clinical sources.[8]

The present study was undertaken to establish a previously unreported industrial source of *Klebsiella* and to compare the biochemical, serological, and mouse pathogenicity characteristics of these *Klebsiella* isolates to those from clinical sources.

MATERIALS AND METHODS

Water samples collected above, below, and at the outfall of the textile finishing plant were transported to the laboratory and maintained at 4°C until they were analyzed, which was usually within 3 hr. Samples were taken on Monday, Tuesday, and Wednesday for 3 consecutive wk.

The numbers of coliform bacteria and the *Klebsiella* portion thereof were measured by a modification of the mC procedure for total and differential coliforms.[15] In the modification, only the *in situ* substrate test for urease activity was used.

The mC medium contained the following ingredients in grams per liter: L-glutamate, 4.0 g; L-cysteine-HCl, 0.5 g; L-proline, 0.4 g; L-tryptophane, 0.3 g; yeast extract, 2.0 g; NH_4Cl, 2 g; lactose, 8.0 g; 500 ml of distilled water; and 500 ml of a salt solution. The salt solution contained, in grams per liter: NaCl, 2.0 g; KCl, 8.0 g; K_2HPO_4, 3.0 g; KH_2PO_4, 1.0 g; and $MgSO_4 \cdot 7\ H_2O$, 0.2 g.

The ingredients were dissolved, the pH adjusted to 7.5 with 10 *N* NaOH, 15.0 g of agar added, and the mixture autoclaved at 121°C for 15 min. Before cooling, 1 ml of 1 percent aqueous solution (w/v) of aniline blue was added. The medium was allowed to stand for 5 min before being cooled to 55°C, after which the following were added in grams per liter: sodium taurocholate, 0.5 g; vancomycin, 0.01 g; and 2,4-dinitrophenol, 10 ml of an 0.1 percent (w/v) aqueous solution. The medium is mixed and dispensed in 4-ml quantities to membrane filter plates.

Appropriate quantities of the samples were passed through 0.45-μm membrane filters, which then were applied to the surface of the mC plates. The plates were incubated at 35°C for 20 to 22 hr. All blue to blue-gray colonies were counted and marked as coliforms by piercing the membrane just below the colony. *Klebsiella* colonies were identified by the *in situ* urease test performed as follows: After the coliforms were counted, the membrane was transferred from the mC plate to an absorbent pad that was saturated with aqueous solution containing 2 percent (w/v) urea and 0.01 percent (w/v) phenol red and that had been adjusted to a pH of 5.2 with 6 *N* HCl. Coliform (blue) colonies that were urease positive took on a deep magenta color, whereas urease-negative coliform colonies remained blue.

Salmonella densities were determined by using the high volume sampling technique of Levin, Fischer, and Cabelli.[16]

The production of indole, acetoin (Voges-Proskauer test), and acid end products (methyl red test), as well as the ability to grow in a salts medium with citrate as the sole carbon source, was tested according to "Standard Methods." [17] Urease ac-

tivity was measured by the 4-hr test of Barry *et al.*[18] Lysine and ornithine decarboxylase and arginine dihydrolase activity were determined by the method of Moller.[19] Fecal coliforms as defined in "Standard Methods"[17] were identified by their ability to grow and produce gas in EC broth at an incubation temperature of 44.5° ± 0.2°C. Motility was observed in SIM medium.*

Serological tests were carried out by using the capillary tube interfacial precipitin test technique.[20] Sixteen pooled and 72 type specific *Klebsiella* antisera were obtained.* Antigens were obtained by growing each isolate in trypticase soy broth overnight at 37°C, centrifuging the culture at 3,100 × g for 15 min, decanting the supernatant, and resuspending the cells in distilled water. One to two drops of 0.1 *N* HCl were added to the cell suspensions before they were boiled for 15 min. The suspensions then were neutralized with dilute KOH and centrifuged at 3,100 × g for 20 min to remove the cell debris. The supernatant was used as the precipitinogen in the serological system. Each antigen was first screened with each of the 16 pools of antisera. Those giving a positive reaction were then tested with the type specific antisera composing the particular pool.

Each isolate to be examined for mouse pathogenicity was grown for 20 hr in trypticase soy broth. The culture was diluted in phosphate-buffered saline (pH 7.2) to a light transmission value of 90 percent in a spectrophotometer. The resulting cell density was approximately 1×10^8/ml. The test was carried out by intraperitoneally injecting each of three 15- to 18-g white mice with 0.5 ml of the bacterial suspension. The mice were observed for 3 days, and the number of deaths was recorded.

RESULTS

Preliminary to the more detailed study of the plant effluents, water samples taken upstream and downstream from the plant and from the plant effluent were analyzed for total coliforms and *Klebsiella*. The average recoveries from three such examinations are shown in Table I. It may be seen that the upstream water contained very few coliforms. The coliform density downstream from the plant was four orders of magnitude greater than that above it. *Klebsiella* made up about 50 percent of the coliform population. The number of coliforms in the plant effluent was six orders of magnitude greater than that found in upstream waters, and more than one-half of these were *Klebsiella*.

TABLE I.—Source of *Klebsiella* in Effluent Receiving Waters

Location	Cells/100 ml*	
	Total Coliforms	*Klebsiella*
Above outfall	300	130
At outfall	2.3×10^8	1.3×10^8
Below outfall	3.7×10^8	1.7×10^8

* As determined from mC procedure.

The plant effluent was further sampled over a 3-wk period to ascertain if the high numbers of *Klebsiella* remained constant from week to week. *Klebsiella* colonies were picked to lactose broth incubated at 35°C for 24 hr. Gas-positive cultures were then transferred to EC broth incubated at 44.5°C. The results of the effluent sampling are shown in Table II. The number of coliforms remained relatively constant over the 3-wk period. The proportion of the total coliforms identified as *Klebsiella* ranged from 43 to 100 percent, with a median percentage of 80. The percentage of *Klebsiella* isolates that gave a positive elevated temperature test ranged from 13 to 70, with a median percentage of 45.

Forty-seven of the isolates were randomly selected for biochemical and serological testing. Pathogenicity studies were conducted on these strains. Thirty clinical isolates were also examined.

The biochemical characteristics of the effluent and clinical isolates are shown in Table III. The characteristics that all of the strains had in common were lactose fermentation, urease activity, decarboxylation of lysine, a lack of ornithine decar-

* Difco, Detroit, Mich.

TABLE II.—*Klebsiella* Densities in Textile Finishing Plant Effluents

Week	Day	Nature of *Klebsiella* in Plant Effluent		
		Density/100 ml × 10^7	Percentage of Total Coliforms*	Percentage of Fecal Coliforms†
1	1	0.6	43	43
	2	80.0	80	No data
	3	130.0	59	70
2	1	0.1	83	70
	2	2.8	68	50
	3	70.0	58	14
3	1	100.0	100	33
	2	25.0	100	45
	3	67.0	89	45

* Obtained as *Klebsiella* density (from column labeled "Density")/total coliform × 100.

† Percentage of *Klebsiella* (from column labeled "Density") that are EC gas positive.

TABLE III.—Characteristics of *Klebsiella* Effluent and Clinical Isolates

Test	Positive Occurrence (%)	
	Effluent (47 strains)	Clinical (30 strains)
Lactose		
Acid	100*	100
Gas	98	97
Indole	29	0
Methyl Red	31	3
Voges-Proskauer	92	99
Citrate	100	100
Urease	100*	97
Lysine decarboxylase	100	100
Arginine dihydrolase	0	0
Ornithine decarboxylase	0	0
H_2S	0	0
Motility	0	0
Gas at 44.5°C	60	83

* Tests used for isolation.

boxylase and arginine dihydrolase activity, and the absence of hydrogen sulfide production. Of the 12 tests carried out, appreciable strain to strain variability was observed with three: the indole, methyl red, and fecal coliform tests.

Because it unfortunately remains a common practice to segregate coliforms by their indole, methyl red, Voges-Proskauer, and citrate (IMViC) reactions, the patterns observed and their frequency of occurrence among the *Klebsiella* isolates are listed in Table IV. Only seven IMViC types were observed. The predominant pattern was the −−++ type, which comprised 63 percent of the effluent isolates and 90 percent of the clinical strains. Eighty-six percent of the effluent isolates had IMViC patterns of −−++, ++++, or −+++. Duncan and Razzell [9] found that 86.6 percent of their environmental isolates were these same three IMViC types. Seven IMViC patterns were represented among the environmental isolates, while the clinical group contained only three IMViC types.

Table V shows the results of the serological testing. Serotypes 1 to 6, those most often associated with respiratory tract infection,[24] made up 35 percent of the effluent isolates. Twenty-four of the possible 72 *Klebsiella* serotypes were encountered in the strains tested. Two of the strains were not reactive.

Very few of the environmental or clinical isolates were pathogenic for mice (killed at least two of three mice injected), even when the injected dose was 1×10^8 viable cells (Table VI). The frequency of mouse pathogenic strains among the effluent isolates was not different from that among the clinical isolates. There was no discernible relationship of mouse pathogenicity to those serotypes commonly associated with human disease.

TABLE IV.—IMViC Patterns of Effluent and Clinical Isolates

Pattern				Positive Occurrence (%)	
I	M	V	C*	Effluent (47 strains)	Clinical (30 strains)
−	−	+	+	62	90
+	+	+	+	15	
−	+	+	+	9	3
−	−	−	+		7
+	−	+	+	6	
+	+	−	+	6	
−	+	−	+	2	

* I, indole; M, methyl red; V, Voges-Proskauer; and C, citrate.

TABLE V.—Serotypes of *Klebsiella* Isolates from Plant Effluents

Serotype	Number Positive	Serotype	Number Positive
1	2	17	1
2	5	18	2
3	2	20	4
4	2	21	1
5	5	23	2
6	1	24	2
8	2	27	1
9	2	31	1
10	1	32	2
13	1	36	1
14	1	44	1
15	2	45	1

DISCUSSION

The practice as described herein of biodegrading polysaccharide-rich textile finishing plant wastes through the use of a fermentation tank-aeration tower system seems to favor the selective multiplication of bacteria of the genus *Klebsiella*. This is especially evident when wastewater has been added as a "seed" for the biodegradation process. In the present study, the interval between "seeding" and the collection of samples was about 2 yr. Because the *Klebsiella* isolates are related to human or animal feces only as the far removed progeny of the "seed," the value of these particular coliforms (some are fecal coliforms) as indicators of the probable presence of enteric pathogens is questionable.

TABLE VI.—Mouse Pathogenicity of *Klebsiella* Isolates

Mouse Response* Dead/Total	Positive Occurrence			
	Effluent (47 strains)		Clinical (7 strains)	
	Number	Percentage	Number	Percentage
3/3	2	4	1	14
2/3	6	13	0	0
1/3	8	16	1	14
0/3	31	65	5	72

* Intraperitoneal injection of approximately 5×10^7 bacteria.

The finding of high densities of *Klebsiella* in textile processing plant wastes is similar to the case of pulp mill and sugar cane wastes, in which the degradation generally is carried out in lagoons rather than in biodegradation tanks and aeration towers.

It has been suggested that coliforms from such industrial effluents still maintain their value as indicators because enteric pathogens found in wastewater, such as *Salmonella*, also are able to "persist" over a period of years in the biodegradation system. This hypothesis was tested in the present study by examining the textile finishing plant effluent for salmonellae by using the HVS technique of Levin, Fischer, and Cabelli.[18] On two separate occasions, 40-l samples of effluent were examined for the presence of *Salmonella*. In both instances none were found, even though the total coliform densities in these samples were about 1×10^6/100 ml. These findings are in agreement with those of Bauer and Knittel,[5, 6] who were not able to demonstrate the presence of *Salmonella* in pulp mill waste effluents that contained coliforms in the millions/100 ml. An indication of the possible fate of *Salmonella* in a similar situation is to be found in the results of Calabra *et al.*[21] They showed that *S. typhimurium* was not able to compete with other bacteria in an artificially seeded septic tank system and that very few salmonellae remained after a period of 2 wk. Gallagher and Spino [22] showed similar die-off rates with *S. typhimurium* inoculated into sugar beet wastes. These findings indicate that, in areas subject to the discharge of textile finishing plant effluents, the finding of high densities of coliforms or even fecal coliforms cannot be used as an index of the probable presence of enteric pathogens. These findings can and do present a problem, however, in that they mask the presence of coliforms from fecal sources.

Klebsiella, however, are pathogens in their own right, although opportunistically so. They are most frequently associated with urinary and respiratory tract infections[23, 24] and are a significant cause of nosocomial disease.[12, 25, 26] The finding of very large numbers of *Klebsiella* in waters

receiving industrial effluents, such as those described herein, requires an evaluation of potential health hazards resulting from their use as a source of direct contact recreation, that is, swimming, water skiing, and so forth. The results of the biochemical and mouse pathogenicity studies indicate that there are no substantial differences in these parameters between effluent and clinical isolates. This would suggest that effluent strains are as hazardous to human health as those actually isolated from clinical cases. This premise must be accepted with a note of caution, however. Mouse pathogenicity may not be an adequate index of the virulence of *Klebsiella* for humans. *Klebsiella* are known to be "opportunists," usually infecting those in a debilitated condition or with some predisposing cause. Therefore, while the pathogenicity to healthy mice of effluent and clinical strains is similar, it does not necessarily follow that both types will have the same effects in humans. Finally, human ID_{50} data, even for clinical isolates, are not available as the basis for making a definitive judgment.

The relatively large numbers of *Klebsiella* serotypes 1 to 6 encountered in this study may have a special health significance in relation to direct contact recreational water activity if industrial effluents are emptied into waters used for this purpose. *K. pneumoniae*, capsular types 1 to 6, have historically been associated with respiratory tract infections. Steinhauer *et al.*[12] studied the capsular types among 181 patients with *Klebsiella* infections. They reported that, although there was no clear-cut association of the capsular type with a specific organ system, capsular types 1, 2, 3, and 5 have a tendency to be associated with respiratory tract infections. Similar findings were reported by Edwards and Fife[24] and by Eisenberg *et al.*,[23] who found that the majority of strains from respiratory tract infections were serotypes 1 to 6 and that these were seldom hospital acquired, as were other serotypes encountered in their study. The high densities of *Klebsiella* that can be found in waters receiving carbohydrate-rich industrial effluents and the relatively high proportion of capsular serotypes 1 to 6 would suggest a hazard to "high risk individuals" if some insult to an individual's respiratory system were incurred during swimming or bathing.

The biochemical characteristics of the effluent isolates contained some interesting differences, even though this group was not dissimilar from the clinical strains in those characteristics that identify the genus (H_2S negative, citrate positive, and ornithine decarboxylase negative). Seven IMViC types were found among the effluent isolates, and three types among the clinical strains. It would seem from these results that there is a selective pressure that tends to segregate for certain IMViC patterns among clinical isolates. Furthermore, when the thermotolerance of these clinical isolates was examined, it was found that all of them were able to grow at 44.5°C. Among the environmental IMViC types, only three contained thermotolerant strains, and two of these types had the same pattern found among the clinical isolates. As indicated in Table VII, of the eight IMViC patterns observed in both types of isolates, the −−++, −+++, −+−+, and −−−+ types showed the thermotolerance characteristic.

It is not readily apparent why all the clinical strains are thermotolerant and fall into only three IMViC patterns. Eickhoff[27] has pointed out that the chance of being infected with *Klebsiella* is greater if

TABLE VII.—Thermotolerance of *Klebsiella* IMViC Types*

IMViC Pattern I	M	V	C	Positive Occurrence: Effluent Strains Number	Effluent Strains Thermotolerant	Clinical Strains Number	Clinical Strains Thermotolerant
−	−	+	+	29	26	27	27
+	+	+	+	7			
−	+	+	+	4	2	1	1
+	−	+	+	3			
+	+	−	+	3			
−	+	−	+	1	1		
−	−	−	+			2	2

* Ability to grow in EC broth at 44.5°C.

an individual's intestine becomes colonized with this organism. Colonization of the intestinal tract has been investigated relative to nosocomial infections. Montgomerie *et al.*[28] attributed colonization of the bowel to food sources, whereas Seldon *et al.*[29] reported that antibiotic therapy predisposed individuals to intestinal colonization with *Klebsiella*. Significantly, both of these studies indicated a relationship between colonization and the incidence of infection. From this it may be assumed that at least certain *Klebsiella* infections in humans are frequently preceded by colonization of the intestinal tract. If most *Klebsiella* infections are preceded by colonization, if colonization requires the organisms to be "fecal" in nature, and if fecal coliforms (as defined by "Standard Methods" [17]) are able to grow at 44.5°C, then the finding that the majority of *Klebsiella* isolates from clinical sources are thermotolerant becomes understandable. Furthermore, if, as suggested by Edwards and Fife [24] and Steinhauer *et al.*,[10] serotypes 1 to 6 are frequently associated with respiratory infections and seldom with nosocomial infections, then it might be expected that the percentage of thermotolerant isolates from respiratory infections would be significantly less than that of other infected organ systems. It would be interesting to examine such strains for the thermotolerant characteristic.

If, as the data suggest, the effluent isolates are not distinguishable from the clinical isolates and, especially, if colonization can take place in waters containing high densities of *Klebsiella*, there also may be an "indirect" hazard associated with the recreational use of such waters. The role that environmental *Klebsiella* plays in causing human infections must, however, in the last analysis be defined by epidemiological means in order that this group may be placed in proper perspective as an environmental health hazard.

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